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# A STUDY OF VIRULENCE AND OTHER PROPERTIES OF DRY PLAGUE CULTURES

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The properties of stored cultures are now stabilized by the widely used method of drying them in a deep vacuum after first freezing and then storing them in a refrigerator. Many authors have investigated the preservation of properties by dried cultures of various species of microorganisms. However, there are few published references to plague microbe cultures and the reports of individual investigators contain conflicting data on the preservation of various plague microbe properties in dried cultures, including virulence (Reitano, 1938; Faybich, 1946; Vasyukhina, 1955; Sokhey, 1958).

The purpose of this study was to check the possibility of a dried plague microbe culture retaining its virulence and some other properties after prolonged storage of the strains in a deep vacuum. Guided by the consideration that the length of time the plague microbe remains virulent on syhthetic nutrient media may vary with the organism of the carrier and the geographic zone in which the cultures may be isolated, we kept under observation 4 strains of the plague microbe: 2 isolated from sick gerbils in Turkmenistan, 1 obtained from a marmot in Kirghizia, and 1 isolated in India from a gray rat.

All 4 cultures were typical strains with respect to morphological and cultural properties. The Dcl for guinea pigs in the strains isolated from the gerbils was 25 m. t. [Russian letters]; in the strains of marmot and rat origin, 100 m. t. The survival time of the dead animals averaged about 6 days for the gerbils and marmots and 9 days for the rats.

The cultures were inoculated on Marten's slant agar(pH 7.2). The condensed water in the flask had to be removed before the culture was inoculated. After 48 hours of growth at 28°, the culture was washed with 3 ml of a stabilizer - 10% sucrose solution with 1.5% gelatin and 0.1% agar (M. M. Faybich). After the concentration was determined, the bacterial suspensions were poured into 0.2 ml ampuls, chilled at -78°, dried in a TsIEM collector apparatus (Dolinov's design) for 17-20 hours, and stored at +2° and +4°. The original control strains were stored on Marten's slant agar in sealed test tubes simultaneously with the dried cultures under the same conditions and times of inoculation. The cultural properties and virulence of the dry and control subcultures stored on agar were studied after 9, 18, 30, and 38 months of storage.

No appreciable changes were noted in the cultural-morphological, biochemical, and serological properties of the dried and control strains throughout the observation period. It will be noted that the dried cultures were much superior to the control in preserving their ability to grow on agar after prolonged storage. The first reinoculation of the dried culture from the ampul on agar plates produced a solid growth of typical viable colonies, whereas in the control, growth during the first generations was sparse and the morphology of the developing colonies was frequently atypical.

As for virulence, 3 of the 4 strains studied lost in varying degree their original virulence. The virulence of subcultures of both the dried and control strains among those isolated in India and Turkmenistan decreased markedly for 18-30 months, but did not wholly disappear. In the strain isolated in India, virulence decreased considerably in the control subculture stored more than 3 years on agar without transfer, less so in the dried culture. The only exception was the strain obtained from Kirghizia (isolated from the marmot). Its virulence scarcely decreased during 38 months of storage either in the dried or control subculture. The latter suggests that plague microbe cultures isolated in Kirghizia preserve their virulence with ordinary storage methods.

Thus, with the above-described regime of drying, the attempt to stabilize the virulence of dry plague microbe cultures failed, but it did produce good results with regard to the preservation of the cultural and morphological properties of the strains.

## Bibliography

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